



THE EFFECT OF STATIN ON ADIPOKINES SECRETION BY VASCULAR ENDOTHELIAL CELLS

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The endothelium plays a crucial role in the maintenance of vascular homeostasis. The increasing incidence of cardiovascular disease associated with diabetes mellitus, obesity or metabolic syndrome significantly enhanced interest in bioactive molecules secreted from adipose tissue (adipocytokines) and their interactions with vascular endothelium. Many research have indicated the abilities of visfatin and resistin to affect and modify endothelial and vascular function. Previous studies indicated that hydroxymethylglutaryl-CoA reductase inhibitors (statins) can reverse endothelial dysfunction in hypercholesterolemic nondiabetic patients. Thus, the aim of the study was to investigate the effect of statin (atorvastatin) on visfatin and resistin secretion by human umbilical vein endothelial cells (HUVECs) during hyperglycemia. It was demonstrated that stable high glucose concentration, the condition that partially mimics glucose excursion in diabetes type II, caused increase in resistin and visfatin release by human endothelial cells. The supplementation of statin caused decrease in visfatin release in dose-dependent manner but did not change resistin secretion. It appears that hyperglycemia-induced increase in visfatin secretion by HUVEC may reduce endothelial dysfunction.

Key words: statin, vascular endothelial cells, resistin, visfatin, hyperglycemia

INTRODUCTION

The endothelium plays a crucial role in the maintenance of vascular homeostasis. The endothelium participates in the regulation of vascular tone and permeability, the composition of the matrix, the balance between coagulation and fibrinolysis, the adhesion of leucocytes, and inflammatory activity in the vessel wall (AVOGARO et al., 2011).

It has been shown that hyperglycemia and glucose fluctuations are strong predictors of cardiovascular diseases (CVD) and microangiopathic damage (GOLDBERG 2003). There is strong evidence for an involvement of hyperglycemia in the vascular impairment in diabetes mellitus, obesity or metabolic syndrome (TESAURO and CARDILLO, 2011). Cardiovascular disease causes most of the excess morbidity and mortality in diabetes mellitus patients. Acute increase of glucose level induces

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oxidative damage, enhancing free radical production, which causes cell apoptosis or necrosis (INOBUCHI et al., 2000). In addition, hyperglycemia directly alters endothelial functions. It has been demonstrated that the endothelial cells exposed to high glucose *in vitro* increase the synthesis of extracellular matrix components (collagen and fibronectin) or procoagulant proteins (von Willebrand, vWF). DUBY et al. (2003) showed that hyperglycemia condition decreased proliferation, migration and fibrinolytic potential of endothelial cells. Furthermore, one of the most important cardiovascular risk factor in type II diabetes is dyslipidemia (MCALLISTER et al., 2010). It is known that diabetes is characterized by increased triglycerides levels which may enhance oxidative stress and impair endothelial function directly and indirectly by increasing the synthesis of small-dense LDL particles and by reducing HDL production (XIE et al., 2010). Previous studies indicated that hydroxymethylglutaryl-CoA reductase inhibitors (statins) can reverse endothelial dysfunction in hypercholesterolemic nondiabetic patients. The mechanism seems to be connected with the upregulation of endothelial NO synthase expression, resulting in increased NO production (MEDA et al., 2010). However, in patients with type 2 diabetes, the results of studies with short-term statin treatment are ambiguous (LI et al., 2005).

The increasing incidence of cardiovascular disease associated with obesity and metabolic syndrome significantly enhances interest in bioactive molecules secreted from adipose tissue (adipocytokines), and their interactions with vascular endothelium (KARASTERGIOU et al., 2010). Recently, ADYA et al. (2008) showed the visfatin-induced production of VEGF and MMP-2 and -9 (metalloproteinases) in both types of human micro- and macrovascular endothelial cells. Furthermore, visfatin may induce migration, tube formation, and angiogenesis possibly through activation of VEGF-MMP pathways (ADYA et al., 2008). Resistin belongs to a family of cysteine-rich secreted polypeptides that are mainly produced by white adipose tissue and monocytes/macrophages (BASTARD et al., 2006). Resistin was found to induce endothelial dysfunction *in vitro*, by increasing the expressions or releases of ET-1, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and pentraxin-3 and decreased the expression of TNF receptor-associated factor-3 (VERMA et al., 2003).

Therefore, the aim of the study was to investigate the effect of statin (atorvastatin) on visfatin and resistin secretion by endothelial cells during hyperglycemia.

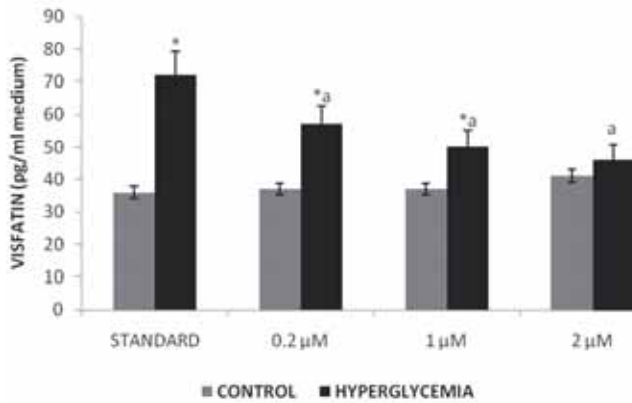
MATERIALS AND METHODS

Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Life Technologies (USA) and grown in endothelial cell growth medium – Medium 200PRF supplemented with LSGS Kit according to the manufacturer's recommendations (Life Technologies, USA). The cells were seeded at the bottom surface of 25-cm² tissue culture flasks and grown at 37°C in 95% air-5% CO₂ to confluence. Culture medium was changed every 24 hours. Subcultures of these cells were used for experiments. Cultured cells were identified as endothelial by their morphology as previously described (JAFFE et al. 1973). HUVECs were seeded at equal density 2.5x 10³cells/cm² and allowed to attach overnight. Endothelial cells between third and sixth passages were taken for experiments. HUVECs were exposed for 10 days to conditions of normal (5mM) and high glucose medium (20mM) with and without atorvastatin (Sigma-Aldrich, USA) at concentrations of 0.2 μM, 1μM and 2μM. After the experiment medium was collected and stored until further analysis.

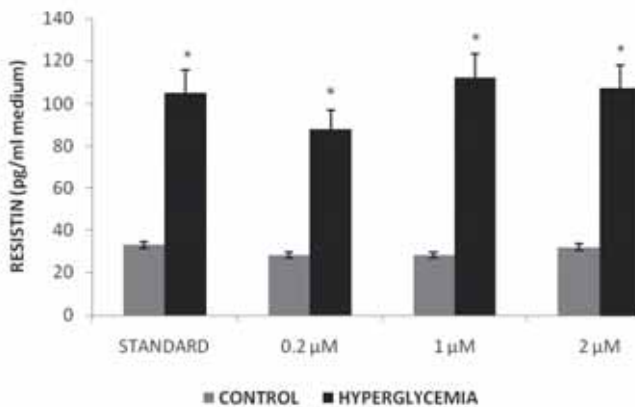
Adipokines assay

Resistin and visfatin concentrations in the culture media were measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Phoenix Pharmaceuticals, USA), according to the manufacturer's instructions. Briefly, the immunoplates in this kits were pre-coated with anti-human-resistin/visfatin capture antibodies and the nonspecific binding sites were blocked. An absorbance of the standard solutions and the experimental samples were measured 20 min after termination of the reaction at 450 nm, using the ELISA plate reader (BioTek, USA). The detection sensitivities for resistin and visfatin was 16 pg/ml and 25pg/ml, respectively.



Standard – without supplementations of statin;
0.2μM, 1 and 2μM statin supplementations

Fig. 1. The effect of atorvastatin on visfatin secretion by HUVECs cultured in different glucose conditions (X±SD, *p<0.01 compared with control; ^a p<0.05 compared with standard)



Standard – without supplementations of statin;
0.2μM, 1 and 2μM statin supplementations

Fig. 2. The effect of atorvastatin on resistin secretion by HUVECs cultured in different glucose conditions (X±SD, *p<0.01 compared with control)

RESULTS

The obtained results indicate that the exposure of HUVECs with high (20mM) glucose concentration significantly changes adipokines secretions. As shown in Figure 1, cells incubated with high glucose showed 2-fold increased secretion of visfatin compared with the control group (p<0.01). The supplementation of atorvastatin decreased visfatin release during hyperglycemia (p<0.05) and its effect was dose - dependent. The highest reduction

of visfatin release was observed after incubation HUVECs with 2 μM atorvastatin (p<0.01).

Analyzing the secretion of resistin in response to hyperglycemia, the experiment showed significantly increased the adipokine level in relation to control group (p<0.01). However, there was no statistically significant differences in resistin secretion after the exposure of endothelial cells to statin.

The experiment was conducted in duplicate. All data were expressed as mean ± SD. Data were analyzed by the Tukey-Kramer test. All statistics was performed using the commercial package StatView (SAS Institute).

DISCUSSION

The study demonstrated that hyperglycemia caused changes of adipokines (resistin and visfatin) secretion pattern in HUVECs. It was shown that stable high glucose concentration, the condition that partially mimics glucose excursion in diabetes type II, caused increase in resistin and visfatin release by human endothelial cells.

Recent studies implicated an important role of visfatin and resistin as both extracellular and intracellular regulators of vascular functions. However, their effect on modulating endothelial cells and smooth muscle cells activities are completely different. The obtained results indicated the significantly increase in resistin secretion in response to hyperglycemia. Moreover, atorvastatin did not have effect on the resistin release. Several previous studies demonstrated that resistin in high glucose increased P-selectin expression, induced monocyte adhesion, generated an increase in NADPH oxidase activity and activated the NFκB by endothelial cells (KOUGIAS et al., 2005). Furthermore, resistin-induced mitochondrial dysfunction contributed to the increase in reactive oxygen species (ROS) which may be the underlying mechanism of endothelial cells damage and smooth muscle cell-mediated remodeling of diseased arteries (CHEN et al., 2010).

BORRADAILE and PICKERING (2009) suggested that increase in visfatin expression in endothelial cells is connected with its vasculoprotective activity. In addition, visfatin seems to decrease cellular oxidative stress by supporting proliferation cells and limiting production of ROS in response to hyper-

glycemia (TAKEBAYASHI et al., 2007). It appears that hyperglycemia-induced increase in visfatin secretion by HUVEC may reduce endothelial dysfunction. Unexpectedly, a supplementation of statin caused decrease in visfatin release in dose-dependent manner. The highest concentration of atorvastatin reversed visfatin release to the basal level. According to clinical trials, statins are prescribed for the prevention of cardiovascular disease even in patients with prediabetes or diabetes, however, their effect on glucose metabolism and the risk of diabetes remains controversial. Some results indicate that the statin therapy, especially an intensive-dose, is associated with a slightly increased risk of development of diabetes (SATTAR et al., 2010).

The present study demonstrated that activities of adipokines (resistin and visfatin) in endothelial cells change in response to hyperglycemia. It appears that an increase in visfatin secretion may be beneficial for endothelium during hyperglycemia. Perhaps monitoring of plasma visfatin levels may be a potential and objective diagnostic marker for cardiovascular disease.

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